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## IN VITRO FERTILIZATION AS A BIOASSAY FOR SCREENING ANTISERA TO NATIVE AND RECOMBINANT ZONA PELLUCIDA (ZP) IMMUNOGEN PREPARATIONS DEVELOPED FOR IMMUNOCONTRACEPTION

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Abstract: Studies to develop immunocontraceptive vaccines for wildlife species often are hampered by reproductive seasonality and limited animal numbers for screening the effectiveness of antigen preparations. The objective of the present study was to determine whether the in vivo immune

response and contraceptive effectiveness of three different zona pellucida immunogens administered to white-tail deer was correlated with the ability of serum antibodies to inhibit sperm-egg binding and fertilization of bovine oocytes in vitro. Prior to the breeding season, groups of eight fertile does were immunized with either 500 μg porcine ZP, (PZP), 600 μg rabbit 55 Kd recombinant ZP protein (RZP-1), 600 µg of the rabbit recombinant 55 Kd protein and two recombinant peptides of the 75 Kd ZP protein (RZP-2) or saline (CON). Initial immunizations were with FCA and followed by boosters at 21 and 51 days using 300 µg of immunogen and FIA. The average numbers of randomly observed matings per doe in each group were PZP (2.0), RZP-1 (0.25), RZP-2 (0.5), and CON (0.5). Immune sera from one or two does in each of the treatment groups were heat inactivated and tested for their ability to inhibit sperm binding and fertilization of bovine oocytes in vitro. Sperm binding to oocytes treated with PZP or RZP-1 antiserum (diluted 1:1) was significantly less than that for those treated with RZP-2 and CON, whereas percent fertilized oocytes was significantly lower only for oocytes treated with PZP antiserum. Given that PZP-treated does were infertile and PZP antiserum from two of the same infertile does inhibited sperm binding and fertilization of bovine occytes in vitro, we suggest that this approach may be useful as a bioassay to screen the potential effectiveness of gamete antigen preparations developed for immunocontraception.